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(54) Fatty acid compositions.

(57) For use in the treatment of disease, a pharmaceutical composition comprises adrenic acid (22:4 n-6 EFA) optionally in combination with γ-linolenic acid or dihomo-γ-linolenic acid and with or without 22:5 n-6 EFA as well.

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"FATTY ACID COMPOSITIONS"

Introduction

The invention relates to fatty acid compositions and their pharmaceutical and dietary uses.

The n-6 or Ω-6 essential fatty acids (EFA's) are required in the body for the structure of membranes in and around cells, being believed to be necessary in particular for maintaining normal flexibility, fluidity and permeability of such membranes. Certain members of the series also act as precursors of prostaglandins (PG's), short-lived regulating agents which modulate many aspects of cellular function.

The pathways of metabolism of the n-6 EFA's and the related n-3 EFA's sharing, it is believed, common emzymes in the two pathways, are:

	n-6		n-3	
$\Delta^{9,12}$	18:2		18:3	$\Delta^{9,12,15}$
	↓	Δ^{6}desaturase	↓	
$\Delta^{6,9,12}$	18:3		18:4	$\Delta^{6,9,12,15}$
	↓	elongation	↓	
$\Delta^{8,11,14}$	20:3		20:4	$\Delta^{8,11,14,17}$
	↓	Δ^{5}desaturase	↓	
$\Delta^{5,8,11,14}$	20:4		20:5	$\Delta^{5,8,11,14,17}$
	↓	elongation	↓	
$\Delta^{7,10,13,16}$	22:4		22:5	$\Delta^{7,10,13,16,19}$
	↓	Δ^{4}desaturase	↓	
$\Delta^{4,7,10,13,16}$	22:5		22:6	$\Delta^{4,7,10,13,16,19}$

The n-3 acids are metabolised preferentially and as a result plasma levels of 18:3 n-3 are low and 18:4 n-3 and 20:4 n-3 are in trace amounts only. In contrast the n-6 acids are all normally detectable, though 18:3 n-6 is at low levels, being

apparently converted to 20:3 n-6 more rapidly than its relatively slow production from 18:2 n-6. The elongation stages in the metabolic pathways are much more rapid than the desaturations.

The acids are in the natural all-cis configurations. In the n-6 series, commonly used names for the 18:2 and 18:3 (octadeca di- and tri-enoic); 20:3 and 20:4 (eicosa tri - and tetra-enoic); and 22:4 (decosatetraenoic) acids are linoleic: acid, γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA), arachidonic acid (AA) and adrenic acid. In the n-3 series only α-linolenic acid (18:3) is commonly referred to by a non-systematic name.

All the n-6 acids are found in plasma and in cell membranes in moderate amounts with the exception of γ-linolenic acid, which is present only in trace quantities because of rapid conversion to dihomo-γ-linolenic acid.

Considering dietary requirements, it is well known that linoleic acid for example cannot be made by the body and so must be taken in the diet. However it has been generally thought that the body can metabolise linoleic acid to all the other n-6 EFA's and therefore that provided linoleic acid intake is adequate, no lack of the other n-6 acids will be found.

In previous patent applications of the present inventor for (example published European Patent Application No. A 0 003 407, U.S. Patent No. 4 273 763; published European Patent Application No. A 0 004 770 U.S. Patent No. 4 309 415; published European Patent Application No. 0 019 423, U.S. Application No. 150 402) it has been pointed out that this is not so and that the first enzyme in the pathway, the Δ-6 desaturase which converts the linoleic acid to γ-linolenic acid, is not fully effective in a variety of conditions. The administration of γ-linolenic acid or dihomo-γ-linolenic acid or both has been suggested in the above patent applications as a way to by-pass this block and has been successful in treating a variety of clinical conditions.

In further studies of the plasma and red blood cells of patients with various diseases the inventor has now found that levels of the C_{22} n-6 acids are reduced from normal in a variety of conditions and the low levels observed are not or not fully corrected by the administration of linoleic acid or, more significantly, of γ-linolenic acid or dihomo-γ-linolenic acid, even in substantial amounts.

The basis for these observations of low C_{22} n-6 acids is believed to be that in many instances there is an excess of 2-series PG production, either alone or coupled with defects of the elongation reaction converting 20:4 to 22:4 and/or the desaturation reaction converting 22:4 to 22:5. The inventor's previous patent applications have pointed out that one way of coping with excess 2-series PG production is selectively increasing the formation of 1-series PG's by, inter alia, ensuring a sufficient supply of γ-linolenic or dihomo-γ-linolenic acid. However, the severe deficiencies of C_{22} n-6 acids that appear to be common in diseases are a new factor. The acids are normally present in all cell membranes, and in high concentration in tissues such as the brain, and a possible reason for the deficiency is excessive consumption of arachidonic acid in conversion to 2-series PG's. Thus whilst C_{22} n-6 acids may be given alone, specific steps are preferably taken to restore 1-series/2-series PG balance, and thus reduce arachidonic acid consumption, by giving γ-linolenic acid and/or dihomo-γ-linolenic acid as well.

The more important of the C_{22} n-6 acids is the 22:4 acid, because the 22:5 acid cannot be converted back to the 22:4 acid in the body and thus the 22:5 acid will be ineffective to restore lack of it, and also because the 22:4 acid, specifically, has recently been found to give rise to homo-2 series PG's in the same way as GLA gives rise to 1-series PG's and arachidonic acid to 2-series PG's. These homo-2 series PG's have as yet been little investigated but may have important functions, requiring administration of 22:4 acid when it is deficient. A failure in the conversion to the 22:5 acid will give a requirement for administration of that acid as well to restore full normality, but if conversion is simply inefficient 22:4 alone may suffice.

It is therefore proposed according to the present invention that in conditions in which deficits of the C_{22} n-6 acids are demonstrated, 22:4,or 22:4 and 22:5 in combination, optionally with γ-linolenic acid and/or dihomo-γ-linolenic acid, should be administered.

Both methods of treatment and compositions of purified natural or synthetic acids in dietary or pharmaceutical vehicles when for such treatment are within the invention, and the acids may be used as such or as pharmaceutically acceptable and physiologically equivalent derivatives as detailed herein. Equivalence is demonstrated by entry into the pathway quoted herein, as evidenced by effects corresponding to those of the acids themselves or their natural glyceride esters. Thus, indirect identification of useful derivatives is by their having the valuable effect in the body of the acid itself, but conversion can be shown directly by gas chromatographic analysis of concentrations in blood, body fat, or other tissue by standard techniques, for example those of Pelick et al. p.23, "Analysis of Lipids and Lipoproteins" Ed. Perkins, American Oil Chemists Society, Champaign, Illinois, U.S.A. Suitable derivatives include those given for γ-linolenic acid and dihomo-γ-linolenic acid later herein; thus reference to any of the acids herein, particularly in the claims, is to be taken as including reference to the acids when in the form of such derivatives.

The significance of the γ-linolenic acid and dihomo-γ-linolenic acid when included is to ensure inter alia that the natural (and irreversible) pathway through to arachidonic acid and onwards is, when its function is impaired rather than inoperative, well supplied with its starting material rather than having to compete for limited supplies with the other pathways, for example, entry of γ-linolenic acid or arachidonic acid into ester storage forms or into PG synthesis, as well as in respect of PG balance as already discussed.

Conditions Treated

The conditions in which the inventor has demonstrated substantial deficiences in the amount of 22:4 or 22:5 acids in plasma or cell membranes include: viral infections, especially with wart viruses; leukaemias, breast cancer and other forms of cancer; premenstrual syndrome and benign breast disease; hypertension, hyperlipidaemias and obesity; the dry eye syndrome, scleroderma, rheumatoid arthritis, Crohn's disease, ulcerative colitis and other forms of auto-immune and inflammatory disorders; male infertility; diabetes; and psychiatric disorders including schizophrenia and alcoholism (including effects both of excess and of withdrawal). It is thus proposed specifically according to the invention to use 22:4,or 22:4 and 22:5 in combination,optionally with γ-linolenic acid or dihomo-γ-linolenic acid in treatment of these conditions. Again the acids may be as such or as derivatives.

Low n-3 Series Levels

In some of the above conditions the n-3 series of EFA's are also at low levels. These conditions are referred to in our co-filed European application No. 84 300 410.2, US application No. 575744 Australian application No 23765/84, Japanese application 14549/84, West German application No. P 3403251.7, Irish application No. 212/84 and Canadian application No.446497. For full treatment of such conditions the compositions of those applications are desirable, counting both n-6 series and n-3 series acids, but n-6 acids predominate generally in the body over n-3 acids, in ratios of, for example, 3:1 or 4:1, so the n-6 acids of the present application will be valuable alone. The reason will be both quantative in the relative amounts and, when the relevant enzymes are defective, leaving them free to act in the one,n-3, pathway only.

Sources of the Acids

Natural sources of 22:4 or 22:5 include adrenal glands (22:5) and kidneys (22:4) obtained from slaughter houses, and 22:4 in the fat of the American Snapping Turtle. Calmic's British specification No. 896903 refers for example to 22:5 in ox adrenal phospholipid

preparations, proposed for use against atherosclerosis, though without discussion of the background with the understanding now available. No reference to 22:4 is made. The acids can be isolated from these sources by, for example, saponification under mild non-oxidising conditions followed by preparative gas liquid chromatography. Synthesis of the acids is difficult but not impossible and provides another source.

Doses

Suggested dose ranges of the 22:4 and 22:5 acids are 5mg to 50g per day, preferably 50mg to 1g per day.

Packs

If it is not desired to have compositions comprising different active materials together, packs may be prepared comprising the materials presented for separate, or part joint and part separate administration in the appropriate relative amounts, and use of such packs is within the purview of the invention.

Dietary Compositions

The invention is chiefly described in terms of pharmaceutical compositions, but it will be understood that the γ-linolenic and other acids, being in the nature of dietary supplements, could be incorporated in a dietary margarine or other foodstuffs; such foodstuffs, possibly containing the other active materials and generally referred to in this description as dietary or pharmaceutical compositions, are within the purview of the invention and thus of the terms pharmaceutical compositions, packs or the like.

Veterinary Applications

It will be understood that where a disorder of a kind calling for treatment in animals arises, the invention whilst described primarily in terms of human medicine and treatment is equally applicable in the veterinary field.

Amounts of γ- and Dihomo-γ-Linolenic Acids

A preferred daily dosage for an adult (weight ca 75 kg) is from 0.05 to 0.1 up to 1, 2, 5 or even 10 g as required for γ-linolenic acid, or equivalent weight (calculated as γ-linolenic acid) of dihomo-γ-linolenic acid or physiologically functional derivative of either. Amounts in particular may be 0.1 to 1.0 g daily. Corresponding doses of Oenothera oil containing 8 to 10% of γ-linolenic acid, are easily calculated.

Forms and Sources of γ-Linolenic and Other Acids

Convenient physiologically functional derivatives of γ-linolenic acid and dihomo-γ-linolenic acid for use according to the invention, as with the 22:4 and 22:5 acids, include salts, amides, esters including glycerides and alkyl (e.g. C_1 to C_4) esters, and phospholipids.

If desired, pharmaceutical compositions may be produced for use in the invention by associating the natural or synthetic acids, as such or as derivatives, with an acceptable pharmaceutical vehicle. It is however at present convenient to incorporate at least the γ-linolenic acid into compositions in the form of an available oil having a high γ-linolenic acid content, hence references to "oil" herein.

At the present time known natural sources of oils having a high γ-linolenic acid content are few (there are no known natural sources of significant amounts of dihomo-γ-linolenic acid). One source of oils currently available is the seed of Evening Primose species such as Oenothera biennis L. and Oenothera lamarckiana, the oil extract therefrom containing γ-linolenic acid (about 8%) and linoleic acid (about 72%) in the form of their glycerides together with other glycerides (percentages based on total fatty acids). Other sources of γ-linolenic acids are Borage species such as Borago officinalis which, though current yield per acre is low, provide a richer source of γ-linolenic acid than Oenothera oil. Recent studies on fungi which can be cultivated by fermentation promise a fungal oil source.

The seed oil extracts referred to above can be used as such or can for example if desired be fractionated to yield an oily composition containing the triglycerides of γ-linolenic and linoleic as the main fatty acid components, the γ-linolenic acid content being if desired a major proportion. Seed oil extracts appear to have a stabilising effect upon dihomo-γ-linolenic acid if present.

PHARMACEUTICAL PRESENTATION

The compositions according to the invention are conveniently in a form suitable for oral, rectal, parenteral or topical administration in a suitable pharmaceutical vehicle, as discussed in detail for example in Williams British Patent Specification No. 1 082 624, to which reference may be made, and in any case very well known generally for any particular kind of preparation. Thus for example tablets, capsules, ingestible liquid or powder preparations, creams and lotions for topical application, or suppositories, can be prepared as required. Injectable solutions of hydrolysed Oenothera oil may be prepared using albumin to solubilise the free acid.

Advantageously a preservative is incorporated into the preparations. α-Tocopherol in concentration of about 0.1% by weight has been found suitable for the purpose.

It will be understood that the absolute quantity of active materials present in any dosage unit should not exceed that appropriate to the rate and manner of administration to be employed but on the other hand should also desirably be adequate to allow the desired rate of administration to be achieved by a small number of doses. The rate of administration will moreover depend on the precise pharmacological action desired.

The following Examples serve to illustrate pharmaceutical compositions useful in treatment according to the invention.

EXAMPLES

Pharmaceutical compositions containing unit doses of one or more of the active materials of the present invention optionally with the oil of the seed of Oenothera biennis L. and optionally further for example with methyl dihomo-γ-linolenate, are presented by encapsulation in soft gelatine capsules by conventional methods.

The Oenothera oil is extracted from the seeds by one of the conventional methods of extraction such as cold pressure, screw pressure after partially cooking the seed, or solvent extraction.

Fractionation of a typical sample of this oil in the form of methyl esters shows the relative proportions:

Palmitate	6.15
Stearate	1.6
Oleate	10.15
Linoleate	72.6
γ-Linolenate	8.9

As preservative, α-tocopherol is added to the oil in a concentration 0.1%.

The following are specific examples of capsules, Examples 1, 2 and 4 optionally also containing 0.5g Oenothera oil, that may be given in the treatment of the conditions listed earlier, 1 to 8 per day (0.5g oil = ca 0.045g γ-linolenic acid)

EXAMPLES

1. Capsules containing 75 or 100mg 22:4
2. Capsules containing 50mg 22:4 and 50mg 22:5
3. Capsules containing 50mg 18:3, in purified form or as in Oenothera oil, and 50mg 22:4
4. Capsules containing 50mg of 20:3 and 50mg of 22:4

Specifically, the capsules may be given against the conditions in respect of which the following tables show normal and diseased n-6 acid levels.

TABLE I. Levels of fatty acids in the plasma phospholipids of control individuals (n,50) and women with cyclical (premenstrual) breast disease (n,21). Levels are expressed as percentages of the total fatty acids present. Each figure represents the mean ± SEM.

Fatty Acid	Control	Cyclical breast Disease
18:2n-6	21.45 ± 0.84	23.26 ± 0.90
20:3n-6	3.06 ± 0.09	2.43 ± 0.11
20:4n-6	11.36 ± 0.24	8.10 ± 0.48
22:4n-6	0.73 ± 0.04	0.23 ± 0.05
22:5n-6	1.12 ± 0.09	0.54 ± 0.07

Corresponding figures for non-cyclical (benign) breast disease, where the low C_{22} n-6 levels are coupled with low n-3 levels also, are:

22.33 ± 0.89
2.54 ± 0.14
7.69 ± 0.36
0.33 ± 0.05
0.73 ± 0.09

TABLE II As Table I. women with premenstrual syndrome signs and symptoms of mood change, fluid retention, weight gain, breast swelling and tenderness, and abdominal distension "Foll" (follicular phase 7-13 days post) "Lut" (late luteal phase, up to 7 days pre). Each figure represents in this and the following Tables the mean ± standard deviation (SD).

Fatty acid	Normal	Premenstrual Foll.	Premenstrual Lut.
18:2n-6	22.74 ± 2.81	25.44 ± 3.46	24.87 ± 3.40
20:3n-6	3.06 ± 0.60	2.79 ± 0.64	2.93 ± 0.44
20:4n-6	11.36 ± 1.67	9.34 ± 1.40	9.26 ± 1.45
22:4n-6	0.73 ± 0.26	0.53 ± 0.33	0.48 ± 0.33
22:5n-6	1.12 ± 0.67	0.36 ± 0.81	0.16 ± 0.35

TABLE III

Fatty acid	18:2n-6	18:3n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6
A. Red blood cell total phospholipids						
Normal + SD	9.78 ± 1.64	-	1.37 ± 0.37	15.13 ± 1.98	5.54 ± 1.37	3.99 ± 1.85
SLE *	15.65 ± 3.84	-	1.91 ± 0.37	14.88 ± 3.43	2.11 ± 0.65	0.27 ± 0.17
Collogenosis	-	-	-	-	-	-
Rheumatoid arthritis	13.30 ± 2.09	-	1.96 ± 0.44	13.21 ± 1.60	2.23 ± 0.59	0.35 ± 0.17
Sjögren's Syndrome	13.69 ± 2.68	-	1.69 ± 0.24	13.69 ± 3.03	2.14 ± 0.65	0.24 ± 0.19
B. Plasma total phospholipids						
Normal + SD	21.45 ± 2.81	0.16 ± 0.12	3.06 ± 0.60	11.36 ± 1.67	0.73 ± 0.26	1.12 ± 0.67
SLE *	23.13 ± 4.72	-	2.65 ± 0.44	11.14 ± 2.47	0.36 ± 0.21	0.09 ± 0.11
Collagenosis	22.19 ± 2.88	0.05 ± 0.10	3.95 ± 0.58	8.18 ± 1.48	0.33 ± 0.19	0.15 ± 0.11
Rheumatoid arthritis	22.19 ± 2.74	-	2.84 ± 0.56	10.62 ± 1.88	0.40 ± 0.32	nd
Sjögren's Syndrome	23.81 ± 2.35	-	2.86 ± 0.71	10.96 ± 1.17	0.19 ± 0.20	0.06 ± 0.14

* Systemic Lupus Erythematosus

nd = not detected

TABLE IV

Plasma Phospholipids

Fatty Acid	Normal	Psoriasis	Asthma *'	Eczema *'
18:2n-6	21.45 ± 2.81	22.66 ± 5.79	21.99 ± 3.31	29.50 ± 3.42
18:3n-6	0.16 ± 0.12	0.05 ± 0.09	0.22 ± 0.31	nd
20:3n-6	3.06 ± 0.60	2.80 ± 0.80	2.96 ± 0.55	2.63 ± 0.52
20:4n-6	11.36 ± 1.67	8.95 ± 1.44	9.18 ± 1.39	6.75 ± 1.12
22:4n-6	0.73 ± 0.26	0.35 ± 0.32	0.14 ± 0.22	0.38 ± 0.24
22:5n-6	1.12 ± 0.67	0.15 ± 0.14	nd	0.13 ± 0.29

*' Coupled with low n-3 acid levels.

Male Infertility	Diabetes
22.6 ± 2.75	23.6 ± 2.94
0.13 ± 0.08	0.08 ± 0.05
3.80 ± 0.96	2.64 ± 0.71
9.56 ± 1.52	9.36 ± 1.24
0.32 ± 0.34	0.42 ± 0.31
nd	0.38 ± 0.24

The method of determination of acid levels was that plasma samples (1 ml) were extracted with chloroform:methanol (2:1). The extract was filtered through sodium sulphate, evaporated to dryness, and taken up in 0.5 ml chloroform:methanol. The lipid fractions were separated by thin layer chromatography on silica gel plates. The phospholipid fraction, taken to reflect essential fatty acid changes most sensitively, was methylated using boron trifluoride-methanol. The resulting methyl esters of the fatty acids were separated and measured using a Hewlett Packard 5880 gas chromatograph with a six foot column packed with 10% silar on chromosorb WAW 106/230. The carrier gas was helium (30 ml/min). Oven temperature was programmed to rise from 165°C to 190°C at 2°C/min. Detector temperature was 220°C and injector temperature 200°C. Retention times and peak areas were automatically computed by Hewlett Packard Level 4 integrator. Peaks were identified by comparison with standard fatty acid methyl esters.

CLAIMS

1. When for use in the treatment of disease, a pharmaceutical composition comprising $\Delta^{7,10,13,16}$ docosatetraenoic acid optionally in combination with γ-linolenic acid or dihomo-γ-linolenic acid.

2. A composition according to Claim 1 comprising also $\Delta^{4,7,10,13,16}$ docosapentaenoic acid.

3. A composition according to Claim 1 or 2, presented for administration to give dose/s of said docosaenoic acid/s of 5 mg to 50 g, preferably 50 mg to 1 g, per day.

4. A composition according to Claim 1 or 2, presented for administration to give dose/s of said linolenic acid/s of 50 mg to 10 g, preferably 100 mg to 1 g, per day.

5. A composition according to Claim 1 or 2 when in the form of a pack comprising the active materials separately, or some separately and others together, but for joint administration.

European Patent Office

EUROPEAN SEARCH REPORT

0132089
Application number

EP 84 30 4610

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
D,E	EP-A-0 115 419 (EFAMOL LTD.) * page 12, lines 1-21; claims 1-4 *	1-5	A 61 K 31/20
X	FR-A-2 231 379 (CENTRE D'ETUDES EXPERIMENTALES ET CLINIQUES DE PHYSIO-BIOLOGIE, DE PHARMACOLOGIE ET D'EUTONOLOGIE) * page 5, lines 1-5; claim 1 *	1-5	
A	GB-A-2 033 745 (THE WELLCOME FOUNDATION LTD.) * page 9, lines 58-62; claim 4 *	1-5	
A	DE-A-3 213 744 (NIPPON SUISAN KAISHA LTD.)	1-5	
A	DE-A-2 749 492 (BIO-OIL RESEARCH LTD.)	1-5	TECHNICAL FIELDS SEARCHED (Int. Cl. ³) A 61 K
D,A	GB-A-1 082 624 (CALMIC LTD.)	1-5	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	18-10-1984	BRINKMANN C.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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